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(71) Applicant *(for all designated States except US)*: FOOD TECHNOLOGY INNOVATIONS PTY. LIMITED [AU/AU]; G13 Biological Sciences Building, University of New South Wales, NSW 2052 (AU).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: WILD, Sylvia [AU/AU]; 170a Parraweena Road, Miranda, NSW 2228 (AU). ELLERMAN, John, Ashley, George [AU/AU]; 5 Arika Close, Bangor, NSW 2234 (AU). TRAN, Lai [AU/AU]; 6 Bradshaw Avenue, Moorebank, NSW 2170 (AU). CONWAY, Patricia, Lynne [AU/AU]; 22 Goorawahl Avenue, La Perouse, NSW 2036 (AU). LUCAS, Rachel, Jane [AU/AU]; 6 Windsor Avenue, Dulwich Hill, NSW 2203 (AU). HENDY, Neil, Andrew [AU/AU]; 65 David Road, Barden Ridge, NSW 2234 (AU).

(74) Agent: F.B. RICE & CO.; 605 Darling Street, Balmain, NSW 2041 (AU).

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(54) Title: A PROCESSED FOOD CONTAINING PROBIOTIC MICROORGANISMS, STARCH/DIETARY FIBRE AND, STABILISERS/EMULSIFIERS

(57) Abstract

An improved processed food product comprising: (a) one or more edible ingredients; (b) a bacterial culture containing one or more probiotic microorganisms; (c) starch containing resistant starch and/or dietary fibre; and (d) one or more stabilisers/emulsifiers; wherein the starch and the one or more stabilisers/emulsifiers act in combination to cause an increased survival rate of the probiotic microorganisms in the food product as compared with a similar food product without the starch and the one or more stabilisers/emulsifiers.

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A PROCESSED FOOD CONTAINING PROBIOTIC MICROORGANISMS, STARCH/DIETARY FIBRE AND, STABILISERS/EMULSIFIERS

Technical Field

The invention relates to processed food products including probiotic microorganisms.

Background Art

Foods are consumed not only for sustenance but also for added health benefits. Examples of such foods include processed milk-based products which have been prepared and consumed by people for centuries. One particular form is the fermented product yoghurt which originally derived from the Middle East Region. Due to extensive marketing, production of low fat varieties, the addition of flavours and fruits, and availability of "off-the-shelf" products, yoghurt has now become a popular product in developed countries. The recent trend of consuming probiotic compositions for health benefits has led to the inclusion of probiotic microorganisms into different processed products, including processed milk-based products. Of these products, yoghurt has been found to be particularly suitable.

As used in this specification, a probiotic or probiotic microorganism is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. This is the definition provided by R. Fuller (AFRC Institute of Food Research, Reading Laboratory, UK) in - Journal of Applied Bacteriology, 1989. 66, pp. 365-378 "Probiotics in Man and Animals - A Review", and has subsequently been extended to include supplements and food for humans.

The constitution and quantity of the gut microflora can be influenced by conditions or stress induced by disease, life style, travel, and other factors. If microorganisms which positively effect the health and well being of the individual can be encouraged to populate the large bowel, this should improve the physiological well being of the host.

The introduction of beneficial microorganisms, or probiotics, is normally accomplished by the ingestion of the organisms in foods, drinks, yoghurts, capsules, and other forms in such a way that the organism arrives in a viable condition in the large bowel.

One problem with the inclusion of probiotic microorganisms into processed food products is that the microorganisms often cannot survive in the food product for any length of time. During production and storage of the food products, there is often a substantial decrease in the numbers of viable

microorganisms. For example, usual shelf-life of milk-based products is calculated on the period of time before spoilage of the product. When probiotic microorganisms are added to these products, the shelf-life stated for the product may not be applicable with regard to delivering the desired number of microorganisms to the gut to obtain the required beneficial effect.

The present inventors have made the surprising discovery that the combination of starch containing resistant starch and/or dietary fibre, and one or more stabilisers/emulsifiers used in processed food products can increase the rate of survival of probiotic microorganisms over the shelf-life of these products.

Disclosure of Invention

In a first aspect, the present invention consists in an improved processed food product comprising one or more edible ingredients, a bacterial culture containing one or more probiotic microorganisms, starch containing resistant starch and/or dietary fibre, and one or more stabilisers/emulsifiers, wherein the starch containing resistant starch and/or dietary fibre and the one or more stabilisers/emulsifiers act to cause an increased survival rate of the probiotic microorganisms in the product.

In a second aspect, the present invention consists in a process for preparing an improved processed food product comprising adding to a food product starch containing resistant starch and/or dietary fibre, one or more stabilisers/emulsifiers, and a bacterial culture containing one or more probiotic microorganisms such that the starch containing resistant starch and/or dietary fibre and the one or more stabilisers/emulsifiers act to cause an increased survival rate of the probiotic microorganisms in the product.

In a third aspect, the present invention consists in a method of prolonging the survival rate of probiotic microorganisms in a processed food product comprising adding to the processed food product starch containing resistant starch and/or dietary fibre, and one or more stabilisers/emulsifiers such that the starch containing resistant starch and/or dietary fibre and the one or more stabilisers/emulsifiers act in the product to cause an increased survival rate of the probiotic microorganisms in the product.

The starch containing resistant starch and/or dietary fibre and the one or more stabilisers/emulsifiers can be added to the product at any stage during the process of producing the improved food product. The present inventors have also found that starch alone can have some beneficial effect

on the survival rate of bacterial cultures when used in food products as well as in probiotic culture preparations. In particular, cultures of probiotic bacteria grown in the presence of starch containing resistant starch and/or dietary fibre and subsequently freeze-dried with the starch for later use were found to have a higher survival rate for the bacteria.

In a preferred embodiment of the first, second and third aspects of the present invention, the processed food product is a fluid-based or solid-based product. Fluid-based food products include milk-based products where the edible ingredient is one or more milk-based ingredients including whole milk, milk solids, milk fat, cream, non-fat dried milk, and any other component or derivative of milk that can be used in milk-based products. Solid-based food products include snack bars, tablets, food additives, health supplements, and pharmaceutical preparations.

The processed food products according to the present invention include any food product that is suitable to contain and deliver probiotic microorganisms. Examples include, but not limited to, fruit beverages, water ices, confectionary, coatings or covertures, yoghurts, yoghurt drinks, unfermented drinks, flavoured milk drinks, modified milk drinks, ice-creams, and dairy desserts. In a preferred embodiment, the product is yoghurt.

Standard methods employed by the art can be used to prepare the processed food products according to the present invention. The starch may be added separately, in combination with one or more of the ingredients that form part of the food product.

The probiotic microorganism is preferably a lactobacillus or bifidobacterium strain, or mixture thereof. Any microbe of gastrointestinal origin or related microbe, however, may also be suitable.

The increase in survival rate of the probiotic microorganisms in the product relates to an increase over the expected survival rate of the same microorganisms in a similar food product that does not contain the combination of starch containing resistant starch and/or dietary fibre and one or more stabilisers/emulsifiers.

The present inventors have found that the inclusion of starch containing resistant starch and/or dietary fibre with one or more stabilisers/emulsifiers currently used in the production of yoghurts resulted in a surprising and unexpected increase in the survival rate of probiotic microorganisms in the yogurt over the shelf-life of the product. There was a

synergistic relationship and/or interaction between the starch containing resistant starch and/or dietary fibre and the one or more stabilisers/emulsifiers in the product. A survival of about 100-fold in numbers of the probiotic microorganisms at the end of one month storage at about 4^{0}C has been detected in yoghurts produced according to the present invention.

In a preferred form, the starch containing resistant starch and/or dietary fibre is a high amylose starch.

The starch containing resistant starch and/or dietary fibre can be used in liquid food products at a concentration of about 0.01 to 10% (w/w) total product. Preferably, the starch is used at 0.1 to 5% (w/w), and more preferably at about 1% (w/w).

The starch containing resistant starch and/or dietary fibre can be used in the dry food products at a concentration of about 0.1 to 90% (w/w) total product. Preferably, the starch is used at about 1 to 10% (w/w).

A further advantage of the use of starch containing resistant starch and/or dietary fibre, and particularly high amylose starches, is that the starch can be added at any stage during the processing of the milk-based product. The properties of the starch are not adversely effected by the processes involved in producing processed milk-based products. One distinct advantage is that there is no need to add the starch in sterile form at the end of the process. The product can undergo pasteurisation or the like without the concern of adversely effecting the starches' properties.

As used in this specification, "dietary fibre and/or resistant starch" includes those forms defined as RS1, RS2, RS3 and RS4 as defined in Brown, McNaught and Moloney (1995) Food Australia 47: 272-275. Either modified or unmodified resistant starches or mixtures thereof can be used in the present invention.

Chemical modifications, such as etherification, esterification, acidification and the like are well known in this art as being suitable chemical treatments. Similarly, other modifications can be induced physically, enzymically or by other means well known to those skilled in the art.

It may also be useful to modify the degree of enzyme susceptibility of the resistant starch by altering the conformation or structure of the starch. Examples include acid or enzyme thinning and cross bonding using di-

functional reagents. Modification of the starch may also be carried out by manipulation of the crystalline nature of the starch. Such modification methods are known to the art and starches produced by these methods would be suitable for the present invention.

Preferably the starches are derived from corn (maize). It will be appreciated, however, that other sources of starch containing resistant starch and/or dietary fibre could be used in the present invention. Examples include cereals like sorghum, wheat, barley and rice, tubers like potatoes and tapioca, legumes such as peas, and others including starches derived from genetically modified plant species.

As used herein, Hi-maize™ refers to a high amylose starch obtained from Starch Australasia Limited and containing over 70% amylose.

Stabilisers/emulsifiers are well known to the art and are used in processed milk-based products to enhance and maintain the desirable characteristics of the product, e.g. body and texture, viscosity/consistency, appearance, and mouth-feel. The classification of food-grade stabilisers/emulsifiers has been made by reference to the processing technique, e.g.

(a) natural gums;

(b) modified natural or semi-synthetic gums; and

(c) synthetic gums.

Examples of each type of stabiliser/emulsifier suitable for use in the present invention are set out in Table 1 (adapted from Tamine and Robertson, Yoghurt Science and Technology 1985, Pergamon Press).

The stabilisers/emulsifiers can be used at a concentration of about 0.1 to 5% (w/w). Preferably the concentration of the stabilisers/emulsifiers is about 0.5 to 2.5% (w/w). It will be appreciated that the concentration can vary depending on the type of product, the amount of high amylose starch containing resistant starch and/or dietary fibre used, and the probiotic microorganisms.

Group 1 "modifying agents" found in the Australian and New Zealand Food Authority (ANZFA) Food Standard A10, either alone or in combination, are suitable for use as the stabilisers/emulsifiers in the present invention.

It has been found that the combination of carrageenan, Locust bean gum (LBG) and type B gelatin is particularly suitable as the stabiliser/emulsifier system for the present invention in processed milk-based products. It will be appreciated, however, that other single or combinations of stabilisers/emulsifiers would also be suitable.

Table 1. Classification and function of gums suitable for milk-based products

Natural gums	Modified gums	Synthetic gums
Plant	Cellulose derivatives	Polymers
Exudates	Carboxymethylcellulose	Polyvinyl derivatives
Arabic	Methylcellulose	Polyvinyl derivative
Tragacanth	Hydroxyethylcellulose	
Karaya	Hydroxypropycellulose	
Extracts	Hydroxypropylmethylcellulose	
Pectins	Microcrystallinecellulose	
Seed flour		
Locust (Carob)		
Guar	Microbial fermentation	
	Dextran	
	Xanthan (β)	
Seaweed		
Extracts		
Agar	Miscellaneous derivatives	
Alginates	Low-methoxy pectin	
Carrageenan	Propylene glycole alginate	
Furcellaran	Pre-gelatinised starches	
Cereal starches	Modified starches	
Wheat	Carboxymethyl starch	
Corn	Hydroxyethyl starch	
	Hydroxypropyl starch	
Animal	Acetylated starches	
Gelatin	Acylated starches	
Casein	Cross-bonded starches	
	Multiple-modified starches	
Vegetable		
Soy protein		

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following drawing and examples.

Brief Description of Drawing

Figure 1 shows the results from Trial 4 demonstrating the effect of Hi-maize™ and stabiliser on counts of *Bifidobacterium* in yoghurt during storage.

Modes for Carrying Out the Invention

Trial 1: Preparation Method 1

The aim of these preliminary trials was to determine the effect of starch containing resistant starch and/or dietary fibre on the survival rate of probiotic microorganisms in processed food products.

Mild and acidic yoghurts were made with A (*Lactobacillus acidophilus*), B (*Bifidobacterium* sp.) and C (*Lactobacillus casei*) cultures plus 0, 1% and 5% Hi-maize™. At the end of 6 weeks storage at 4°C the following observations were made:-

- 'A' count was improved by a factor of 10 by the addition of 1% or 5% Hi-maize™ and counts were maintained at 10^0 cfu/g
- Hi-maize™ had a beneficial effect towards the survival of *Bifidobacterium* sp. DS920 up to 4 weeks, maintaining counts of 10^5 to 10^6 cfu/g (cf., $<10^4$ in absence of Hi-maize™). However at 6 weeks all yoghurts contained $<10^4$ cfu/g.
- 'C' counts were maintained at 10^7 cfu/g.

Trial 2

Repeat work has shown that Hi-maize™ is effective in assisting the survival of A and B cultures in yoghurt.

Trial 3

Repeat work showed that Hi-maize™ maintained the viability of B at least 1 order of magnitude when compared with control, particularly during the last 2 weeks of shelf-life storage.

A combination of carrageenan, Locust bean gum (LBG) and type B gelatin was used as the stabiliser/emulsifier system for the following Trials.

No significant differences were observed between the two methods of yoghurt preparation and shearing although the Method 2 of preparing yoghurt base may assist initial survival of Bifidobacteria. The *acidophilus* counts were generally maintained at levels of 10^7 cfu/g to 5 weeks and held this level when in the presence of Hi-maize™ and/or stabiliser to the end of shelf-life. Final shelf-life results are summarised in the table below. The most interesting result from this experiment was the effect the stabiliser (plus Hi-maize™) had on the survival of Bifidobacteria. These results are also summarised in the table below. All trials containing stabiliser and Hi-maize™ maintained Bifidobacteria counts of 10^6 cfu/g at 6 weeks.

Table 2. General trends for end of shelf-life counts at 6 weeks for A and B cultures (inoculation at 10U/1000L) in mild yoghurt

Yoghurt Type	*acidophilus* (cfu/g)	Bifidobacteria (cfu/g)
+ Hi-maize™ + Stabiliser	10^7	10^6
- Hi-maize™ + Stabiliser	10^7	10^4
+ Hi-maize™ - Stabiliser	10^5 - 10^7	10^3-10^4
- Hi-maize™ - Stabiliser	10^5	10^3

Trial 4: Comparison of Preparation Method 1 and Method 2

A trial assessing yoghurt base heat-treatment methods, yoghurt shearing methods and presence/absence of stabiliser and Hi-maize™ was carried out. This work was carried out to try and determine the difference between Method 1 and Method 2 results re: probiotic counts.

Hi-maize™ was used in yoghurt makes to study its efficacy in assisting the survival of probiotic cultures contained in yoghurt, especially *Bifidobacterium* sp. cultures. Based on the results of initial trials 1 to 3, a further trial was set up that included the preparation processes of Method 1 and Method 2 in order to confirm the support of Hi-maize™ and stabilisers (mixture of carrageenan, Locust bean gum (LBG) and type B gelatin) towards the survival of the probiotic cultures in yoghurt.

METHODS

Yoghurt Base (applicable for all trials)

Using standard base as in the previous trials ie: 14% total solids non-fat (TSNF), 1.2% fat (F) and 6% sugar, with the following combination of Hi-

maize™ and stabiliser (mixture of carrageenan, Locust bean gum (LBG) and type B gelatin):

base 1 : - Hi- Maize + Stabiliser
base 2 : + Hi-Maize + Stabiliser
base 3 : - Hi- Maize - Stabiliser
base 4 : + Hi- Maize - Stabiliser

1% Hi-maize™ and 1.8% stabiliser were used and these bases were heat treated by:

- Method 1 preparation: 90°C for 10 mins
- Method 2 preparation: 65°C for 3 mins, stir, then heat to 92°C, and hold for 6 mins.

Inoculum Level (applicable for all trials)

Using similar inoculum level of all cultures as in the previous trials:

C 4U/1000L
A 10U/1000L
B 10U/1000L

Incubation Temperature

43°C

Strike pH

4.5

Shearing Method

After incubation, the yoghurts were divided into two loads for two different methods of shearing:

- Method 1 shearing: shearing by pumping through 0.5 mm screen
- Method 2 shearing: stirring with paddle

There were 16 samples to analyse the shelf-life of A and B cultures over 6 weeks

1Aa, 1Ab and 1Ba, 1Bb
2Aa, 2Ab 2Ba, 2Bb
3Aa, 3Ab 3Ba, 3Bb
4Aa, 4Ab 4Ba, 4Bb

Formulation Notes:

Yoghurt 1 : - Hi-maize™ + Stabiliser base
Yoghurt 2 : + Hi-maize™ + Stabiliser base

Yoghurt 3 : - Hi-maize™ - Stabiliser base

Yoghurt 4 : + Hi-maize™ - Stabiliser base

A : Method 1 of treat treatment

B : Method 2 of heat treatment

a : Method 1 of shearing

b : Method 2 of shearing

Starch Protection Study

Bifidobacterium sp. DS920 was cultured in the presence or absence of Hi-maize™ to prepare bacterial cultures containing probiotic microorganisms for addition to food products. The cultures were then freeze-dried by standard techniques for subsequent use. Viability/stability tests were carried out by placing samples of the cultures in sealed containers and holding the containers at 40°C for up to a week. Samples were taken at intervals and the number of viable bacteria calculated.

RESULTS

1) Acidophilus results

Generally, *L. acidophilus* DS910 seemed to maintain well ($>10^6$ - 10^7 cfu/g at 5 weeks) in all yoghurts with added Hi-maize™ and/or stabiliser. In the control yoghurt 3 (ie - Hi-maize™ - stabiliser), the A count started to drop from 10^7 to 10^6 cfu/g from week 3, then further to 10^5 cfu/g at week 6 in yoghurt prepared by Method 2. Yoghurt prepared by the Method 1 maintained an A count of 10^7 cfu/g until week 5 then dropped to 10^5 at the end. *Acidophilus* counts in the Method 2 prepared yoghurt with Hi-maize™ also dropped to 10^5 at 6 weeks which compares with Method 1 prepared yoghurt plus Hi-maize™ which maintained counts of 10^7 cfu/g.

2) Bifidobacteria results

The shelf-life results of *Bifidobacterium* sp. DS920 during 6 weeks storage in mild yoghurt at 4- 5°C are summarised in Figure 1. Without Hi-maize™ and stabiliser, the Bifidobacteria population dropped from about 10^7 cfu/g to about 10^3 cfu/g at the end of 6 weeks for both methods (yoghurt 3) (Figure 1).

Yoghurt containing both Hi-maize™ and stabiliser (yoghurt 2) retained a Bifidobacteria count of 10^6 cfu/g at 6 weeks.

The stabiliser alone appears to give more protection to the Bifidobacteria population than Hi-maize™ alone in yoghurt as the B count in yoghurt 1 (-Hi-maize™, stabiliser) was 10^5 cfu/g at 4 week-storage compared

to the B count in yoghurt 4 (+Hi-maize™,- stabiliser) which was only 10^4 cfu/g. This finding was consistent for all trials carried out. The surprising result was the synergistic effect of the combination of Hi-maize™ and stabiliser on survival of Bifidobacteria. Although both Hi-maize™ and stabiliser alone have some beneficial effect, the microbial survival rate detected from the synergistic combination of Hi-maize™ and stabiliser in yoghurt was significantly higher than the expected result from the results of the individual effects of Hi-maize™ and stabiliser when used alone.

Although yoghurt was used in the trials described above, it will be appreciated that other processed food products containing probiotic microorganisms may also be prepared using the synergistic combination of starch containing resistant starch and/or dietary fibre and stabilisers/emulsifiers to enhance the survival rate of the probiotic microorganisms during the product's shelf-life.

Starch Protection Study

The results of the viability/stability tests of freeze-dried cultures of *Bifidobacterium* sp. DS920 in the absence and presence of Hi-maize™ are shown in Table 3. As can be seen from the results obtained, the growth and storage of bacteria in the presence of Hi-maize™ greatly enhanced the survival rate of the bacteria. A number of other bacterial strains were also tested under the same conditions and similar survival rates were obtained.

Table 3. Survival of *Bifidobacterium* sp. DS920 in freeze-dried cultures

Days	DS920 - Hi-maize™ (Units cfu/g dried culture)	DS920 + Hi-maize™ (Units cfu/g dried culture)
0	3.3×10^{10}	3.90×10^{10}
1	2.00×10^{10}	1.70×10^{10}
2	1.30×10^{9}	1.30×10^{10}
3	1.80×10^{7}	1.00×10^{10}
4	4.00×10^{4}	3.00×10^{9}
7	0	1.10×10^{8}

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:

1. An improved processed food product comprising:

(a) one or more edible ingredients;

(b) a bacterial culture containing one or more probiotic microorganisms;

(c) starch containing resistant starch and/or dietary fibre; and

(d) one or more stabilisers/emulsifiers;

wherein the starch and the one or more stabilisers/emulsifiers act in combination to cause an increased survival rate of the probiotic microorganisms in the food product as compared with a similar food product without the starch and the one or more stabilisers/emulsifiers.

2. The processed food product according to claim 1 being selected from the group consisting of fruit beverages, water ices, confectionary, coatings or covertures, yoghurts, yoghurt drinks, unfermented drinks, flavoured milk drinks, modified milk drinks, ice-creams, dairy desserts, snack bars, tablets, food additives, health supplements, and pharmaceutical preparations.

3. The processed food product according to claim 2 being a milk-based food product.

4. The processed food product according to claim 3 wherein the one or more edible ingredients are selected from the group consisting of whole milk, milk solids, milk fat, cream, non-fat dried milk, and any other component or derivative of milk.

5. The processed food product according to claim 3 being yoghurt.

6. The processed food product according to any one of claims 1 to 5 wherein the probiotic microorganism is a lactobacillus strain, bifidobacterium strain, or mixture thereof.

7. The processed food product according to any one of claims 1 to 6 wherein the starch is derived from the group consisting of cereals including corn, sorghum, wheat, barley and rice, tubers including potatoes and tapioca, legumes such as peas, and other starches derived from genetically modified plant species.

8 The processed food product according to claim 7 wherein the starch is derived from corn.

9. The processed food product according to claim 8 wherein the starch is modified or unmodified high amylose corn starch having at least 70% (w/w) amylose.

10. The processed food product according to any one of claims 1 to 9 wherein the starch is used in a fluid-based product at a concentration of 0.01 to 10% (w/w) total product.

11. The processed food product according to claim 10 wherein the starch is used at 1% (w/w) total product.

12. The processed food product according to any one of claims 1 to 9 wherein the starch is used in a solid-based product at a concentration of 0.1 to 90% (w/w) total product.

13. The processed food product according to any one of claims 1 to 12 wherein the stabilisers/emulsifiers are selected from the group consisting of natural gums, modified natural or semi-synthetic gums, synthetic gums, and mixtures thereof.

14. The processed food product according to claim 13 wherein the stabilisers/emulsifiers are a combination of carrageenan, Locust bean gum, and type B gelatin.

15. The processed food product according to any one of claims 1 to 14 wherein the stabilisers/emulsifiers are used at a concentration of 0.1 to 5% (w/w) total food product.

16. The processed food product according to claim 15 wherein the concentration of the stabilisers/emulsifiers is 0.5 to 2.5% (w/w) total food product.

17. The processed food product according to any one of claims 1 to 16 wherein the starch is added to the food product in association with the bacterial culture.

18. Use of a synergistic combination of starch containing resistant starch and/or dietary fibre and one or more stabilisers/emulsifiers in a processed food product including a bacterial culture containing one or more probiotic microorganisms to cause an increased survival rate of the probiotic microorganisms in the food product as compared with a similar food product without the starch and the one or more stabilisers/emulsifiers.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00557

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A23L 1/0522 3/3463 A23C 9/137 A61K 35/72 35/74 35/78 47/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT: (A23C/IC or A23L/IC) and (Probiotic or microorganism# or microb: or bacteria: or lactobacillus or bifidobacterium) and (starch: or fiber# or fibre#) and (stabili: or emuslif:)
CAS: (starch(3n)resistant) and (lactobacillus or bifidobacterium)
(17/cc or 17/sx) and (dietary fiber/ct and lactobacillus or bifidobacterium)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 806 368 (MALIREDDY REDDY) 21 February 1989 Column 1 lines 16-17	1-18
P,X	WO 97/34615 (THE UNIVERSITY OF NEW SOUTH WALES et al.) 25 September 1997 Page 6	1-18
P,X	WO 97/34592 THE UNIVERSITY OF NEW SOUTH WALES et al.) 25 September 1997 Page 4	1-18

[X] Further documents are listed in the continuation of Box C

[ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 August 1998	25 AUG 1998
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer MR LEIGH R. TRISTRAM Telephone No.: (02) 6283 .

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00557

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 97/34591 (UNIVERSITY OF NEW SOUTH WALES) 25 September 1997 Page 9 Table 1, page 11 Table 4, page 17 example 6 Page 25 Table 14	1-18
X	WO 96/08261 (UNIVERSITY OF NEW SOUTH WALES et al) 21 March 1996 Page 3 lines 5-12, page 5 lines 28-29 Page 6 lines 31-34, page 8 line 28 - page 9 line 7 Page 13 Table 3, page 15 - page 22	1-18
X	WO 91/17672 (HANNU SALOVAARA and ANNE-MARIE KURKA) 28 November 1991 Pages 6-11	1-4, 6, 7, 10, 12, 18
X	EP 399 819 (TOYO JOZO KABUSHIKI KAISHA) 28 November 1990 Examples 1-13	1-18
X	AU 81807/94 (SOCIETE DES PRODUITS NESTLE S.A.) 20 July 1995 Whole document	1-7,10-18
X	US 4797289 (MALIREDDY REDDY) 10 January 1989 Column 2 lines 63-66 Column 5 lines 24-29 and examples	1-4,6,7,10,12,

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 98/00557

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9734615	AU	20182/97				
WO	9734592	AU	20181/97				
WO	973591	AU	20180/97				
WO	9608261	AU	35579/95	CA	2199140	EP	778778
WO	9117672	AU	78702/91	EP	568530	FI	902464
EP	399819	CA	2017339	PT	94108	US	5283059
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		EP	667106	JP	7231750	NO	950085
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END OF ANNEX